

**AMENDMENT AFTER FINAL  
U.S. Appln. No. 09/830,876**

**IN THE CLAIMS:**

**This listing of claims will replace all prior versions and listings of claims in the application:**

**LISTING OF CLAIMS:**

Claim 1. (Previously Presented) A two-site immunoassay for the qualitative or quantitative detection of alpha-amylase in a test sample, said immunoassay comprising;

- (i) exposing said test sample to a first antibody or fragment thereof which specifically or preferentially binds to a first epitope on said alpha-amylase, under conditions permitting binding of said first antibody or fragment thereof to alpha-amylase if present,
- (ii) subsequently exposing bound alpha-amylase, if any, to a second antibody or fragment thereof which specifically or preferentially binds to a second epitope on said alpha-amylase that is distinct from said first epitope, under conditions permitting binding of said second antibody or fragment thereof to said bound alpha-amylase, and
- (iii) detecting any binding of said second antibody or fragment thereof to said bound alpha-amylase,

wherein either of said first or second epitopes is an epitope comprising one or more amino acid sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, and wherein detection of binding of said second antibody or fragment thereof to said bound alpha-amylase

**AMENDMENT AFTER FINAL**  
**U.S. Appln. No. 09/830,876**

qualitatively or quantitatively indicates the presence of alpha-amylase in said test sample.

Claim 2. (Cancelled).

Claim 3. (Previously Presented) The immunoassay according to claim 1, wherein either of said first or second epitopes is a conformational epitope comprising one or more of the amino acid sequences; IDRLVSIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ ID NO: 2), VNWVNKVGGG (SEQ ID NO: 3).

Claim 4. (Previously Presented) The immunoassay according to claim 1, wherein either of said first or second epitopes is a conformational epitope comprising all of the amino acid sequences; IDRLVSIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ ID NO: 2), VNWVNKVGGG (SEQ ID NO: 3).

Claim 5. (Previously Presented) The immunoassay according to claim 1, wherein said first antibody or fragment thereof or said second antibody or fragment thereof is bound to a solid support.

Claim 6. (Previously Presented) The immunoassay according to claim 5, wherein the solid support is selected from microwell plates, membranes, beads, particles, sensors and porous test strips.

Claim 7. (Previously Presented) The immunoassay according to claim 1, wherein binding of the second antibody or fragment thereof to alpha-amylase is detected through the use of a readily detectable label.

Claim 8. (Previously Presented) The immunoassay according to claim 7, wherein the detectable label is selected from detectable enzymes, radioisotopes, luminescent labels and fluorescent labels.

Claim 9. (Previously Presented) The immunoassay according to claim 1, wherein binding of the second antibody

**AMENDMENT AFTER FINAL**  
**U.S. Appln. No. 09/830,876**

or fragment thereof to alpha-amylase is detected through the use of immunochromatography or agglutination.

Claim 10. (Previously Presented) The immunoassay according to claim 1, wherein at least one of the first and second antibodies or fragments thereof is a monoclonal antibody or fragment thereof.

Claim 11. (Previously Presented) The immunoassay according to claim 1, wherein the test sample is obtained from a cereal grain.

Claim 12. (Previously Presented) The immunoassay according to claim 11, wherein the cereal grain is selected from the group consisting of bread wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum* var. durum), club wheat (*Triticum compactus*), rye (*Secale cereale*), triticale (*Triticosecale* species) and barley (*Hordeum vulgare*).

Claim 13. (Previously Presented) The immunoassay according to claim 11, wherein the test sample is an extract from grain, grain meal or flour in an aqueous extraction medium, and optionally comprised NaCl or CaCl<sub>2</sub>.

Claim 14. (Previously Presented) The immunoassay according to claim 1, wherein said immunoassay provides for the quantitative detection of alpha-amylase by further comprising:

- (iv) comparing the level of detected binding of the second antibody or fragment thereof for the test sample to the levels of detected binding of said second antibody or fragment thereof to samples having known alpha-amylase enzyme activities, or Falling Numbers thereby providing for quantitative detection of alpha-amylase in said test sample.

**AMENDMENT AFTER FINAL**  
**U.S. Appln. No. 09/830,876**

Claims 15-22. (Cancelled).

Claim 23. (Previously Presented) The immunoassay of claim 1, wherein the test sample comprises grain meal or flour.

Claim 24. (Previously Presented) The method of claim 13, wherein the aqueous extraction medium comprises NaCl or CaCl<sub>2</sub>.

Claim 25. (Previously Presented) A process for determining weather damage in a plant or crop said process comprising performing the method of claim 1 on one or more test samples selected from the group consisting of grain, grain meal, flour, an aqueous extract of grain, an aqueous extract of grain meal and an aqueous extract of flour, wherein said test sample is obtained from said plant or crop and wherein the presence of alpha-amylase in said test sample as determined by the level of detected binding of the second antibody or fragment thereof to the test sample indicates that the plant or crop has been weather damaged.

Claim 26. (Previously Presented) The process of claim 25, wherein the test sample is obtained from a cereal grain.

Claim 27. (Previously Presented) The process of claim 26, wherein the cereal grain is selected from the group consisting of bread wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum* var. durum), club wheat (*Triticum compactus*), rye (*Secale cereale*), triticale (*Triticosecale* species) and barley (*Hordeum vulgare*).

Claim 28. (Previously Presented) The process of claim 25, wherein the test sample is an aqueous extract from grain, grain meal or flour.

Claim 29. (Previously Presented) The process of claim 25, further comprising quantifying the amount of alpha-amylase in the test sample by a process comprising comparing the level of

**AMENDMENT AFTER FINAL**  
**U.S. Appln. No. 09/830,876**

detected binding of the second antibody or fragment thereof for the test sample to the levels of detected binding of said second antibody or fragment thereof to samples having known alpha-amylase enzyme activities, viscosities, or Falling Numbers thereby quantifying the amount of alpha-amylase in said test sample.

Claim 30. (Previously Presented) The immunoassay of claim 1, wherein said immunoassay provides for the qualitative detection of alpha-amylase by further comprising:

- (iv) comparing the level of detected binding of the second antibody or fragment thereof for the test sample to the levels of detected binding of said second antibody or fragment thereof to samples having known viscosities thereby providing for quantitative detection of alpha-amylase in said test sample.